

# Toepassing van circulerend, cel-vrij DNA in plasma als 'liquid biopsy' voor solide tumoren

PAUL-EMILE CLAUS  
ASO KLINISCHE BIOLOGIE, AZ DELTA

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DR. DIETER DE SMET



1. *De rol van moleculaire diagnostiek bij de keuze van **precisie therapie bij NSCLC**.*
2. *Wat is **cel-vrij, circulerend tumor DNA (ctDNA)** en hoe kan de analyse hiervan een meerwaarde betekenen voor de diagnostiek en opvolging van NSCLC patiënten? Wat is er gekend over de stabiliteit en kritische (pre-) analytische factoren van ctDNA?*
3. *Welke **moleculaire assays** kunnen gebruikt worden voor de **detectie van ctDNA** en met welke technische aspecten moet men rekening houden?*

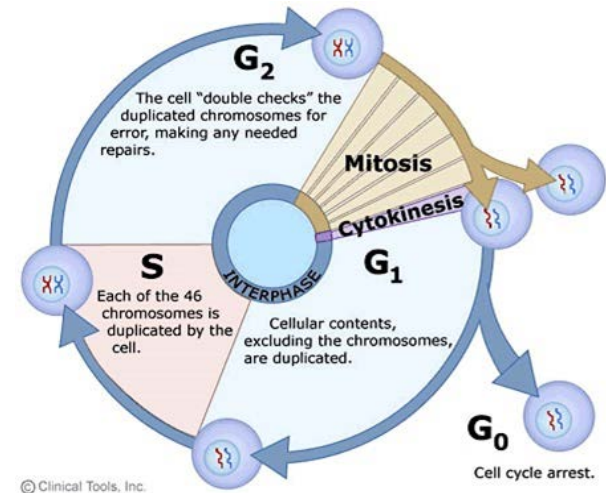


*Klinische meerwaarde van ctDNA?*



## Tumoren: ontregeling in de celcyclus

- Angeboren (germline) DNA mutaties
  - BRCA1, BRCA2: borst
- Verworven (somatische) puntmutaties
  - EGFR signalisatie (MAPK-pathway): long, colon, melanoma
  - P53 checkpoint
- Gain-of-function vs. Loss-of-function mutaties
- Structurele afwijkingen: puntmutaties, inserties, deleties, translocaties, CNV, methylaties



Ongecontroleerde celdeling of proliferatie

# Clinical/Diagnostic Scenario



No. 22/September 13, 2011  
**Chancellor Angela Merkel Presses Start Button for New State-of-the-Art Sequencer at the Max Delbrück Center in Berlin**

Extended Potential for Medical Systems Biology

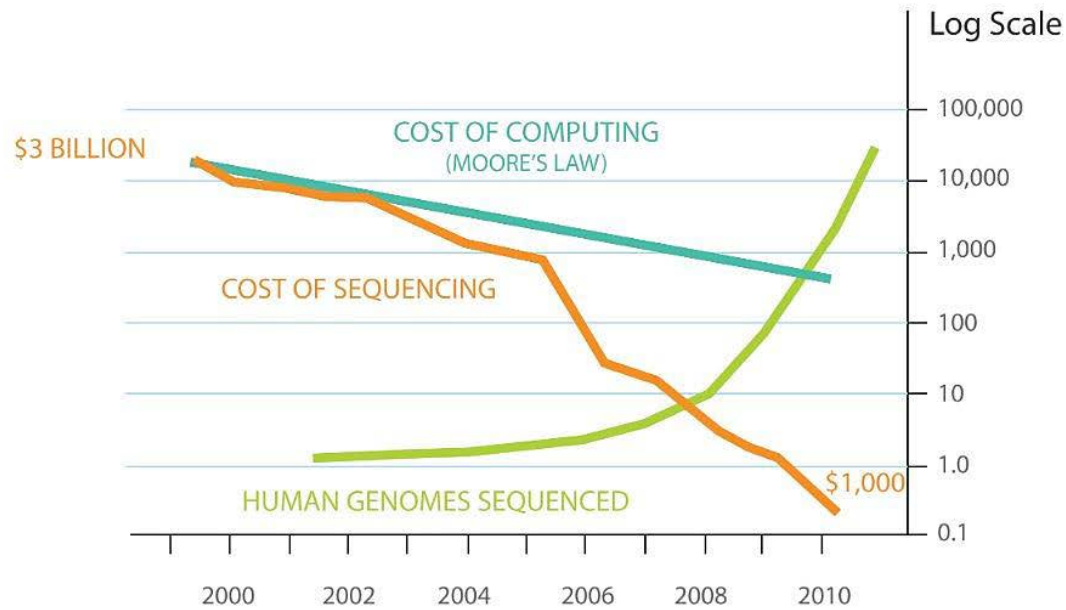


### WHY NOW?

The **time is right** because of:

- Sequencing of the human genome
- Improved technologies for biomedical analysis
- New tools for using large datasets

## THE SEQUENCING EXPLOSION



illumina



ion torrent  
♦ ★ ▲ ○ × □ + ∞



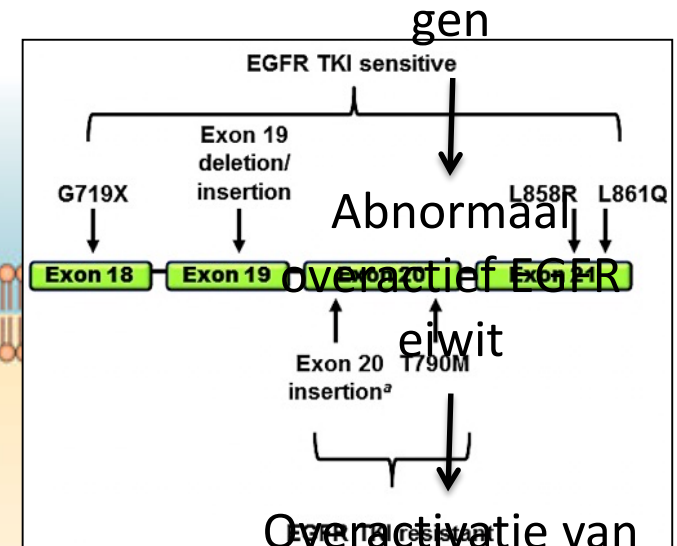
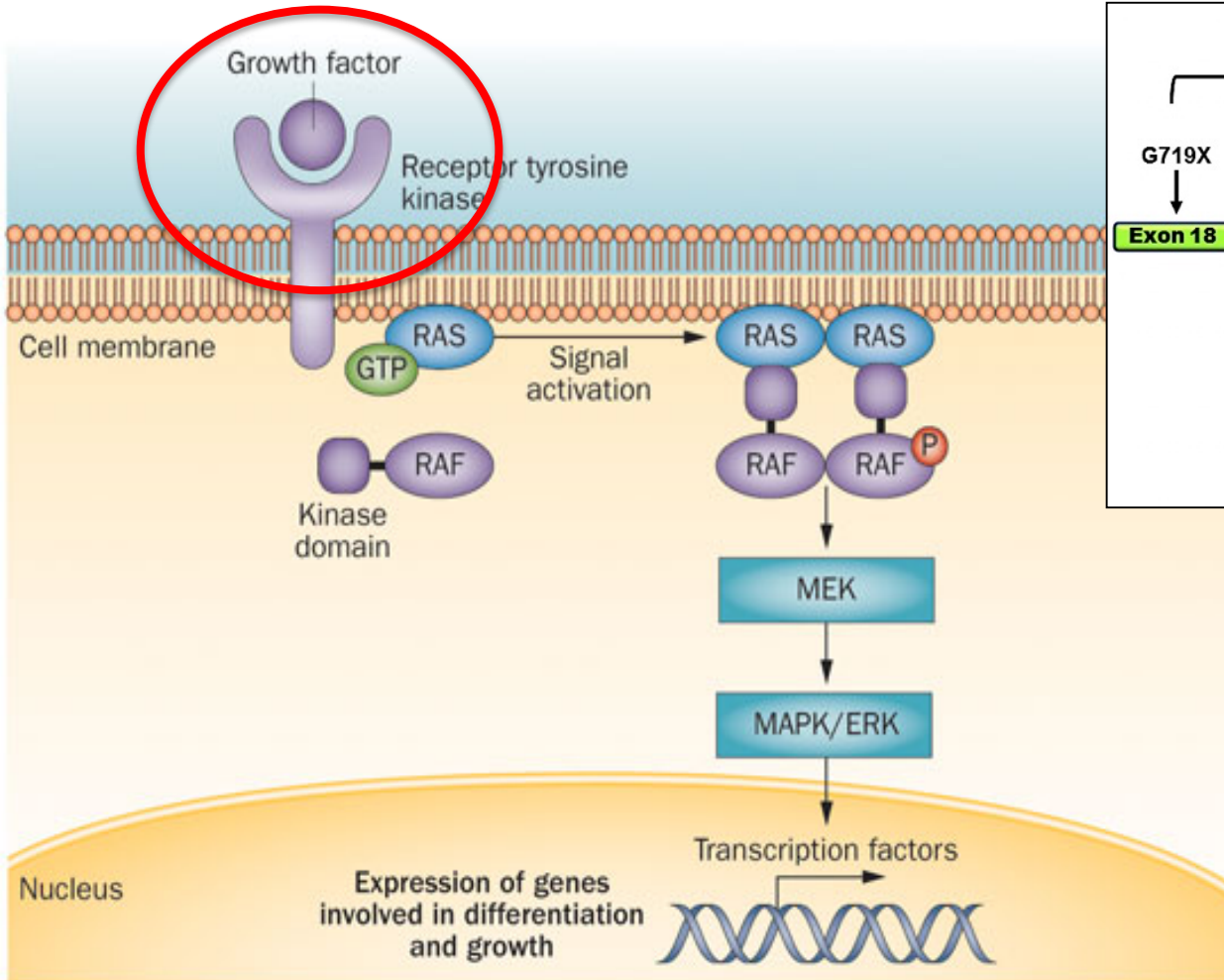
Roche

1. *De rol van moleculaire diagnostiek bij de keuze van **precisie therapie bij NSCLC**.*
  1. *Welke zijn de belangrijkste, therapeutisch relevante somatische mutaties ( ‘actionable mutations ’) waarvoor specifieke testen ( ‘companion diagnostics ’) noodzakelijk zijn in de huidige praktijkvoering?*
  2. *Wat zijn de beperkingen van de huidige state-of-the-art analyse van longweefselbiopten?*



## Groefactoren stimuleren de celdeling: EGFR

L858R mutatie EGFR



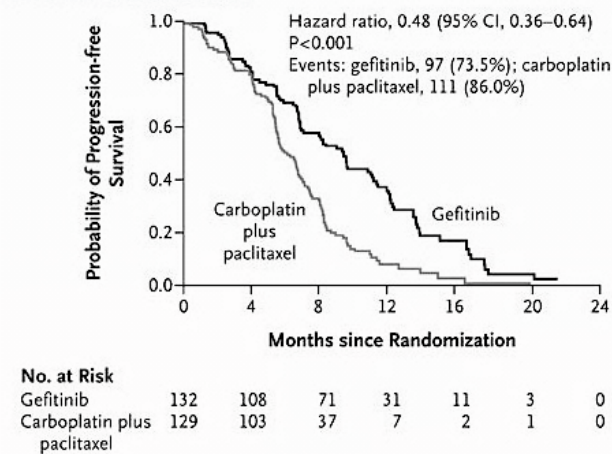
Overactivatie van  
MAPK-pathway

Ongecontroleerde  
celdeling

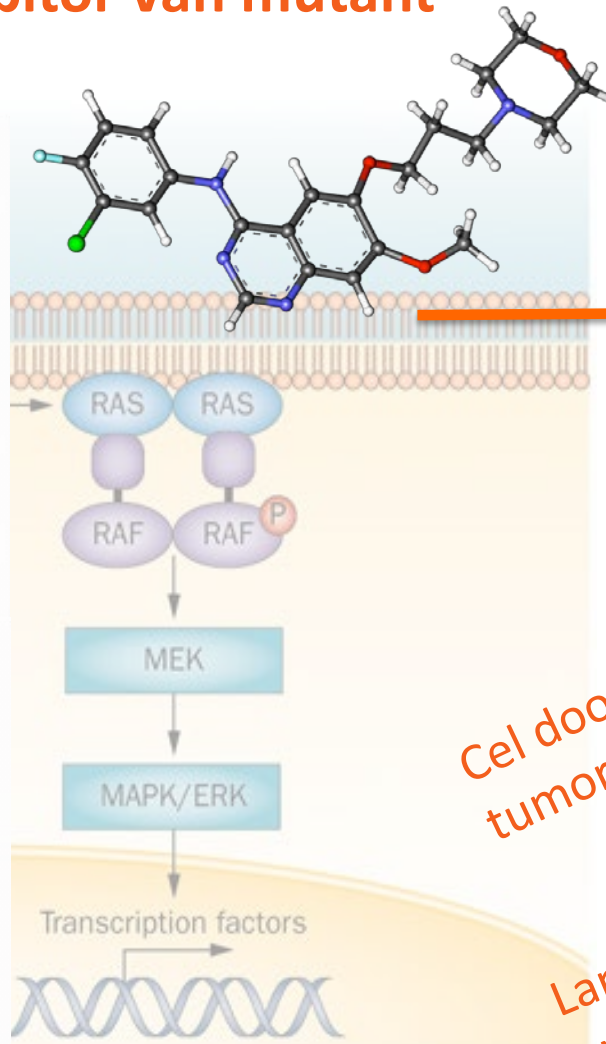
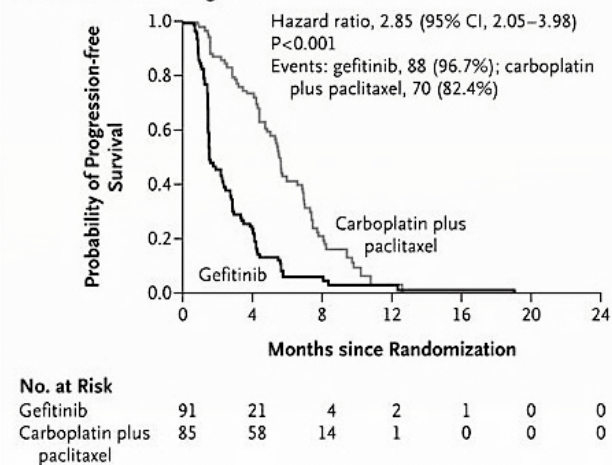
Longkanker

## Gefitinib: specifieke inhibitor van mutant EGFR

**B EGFR-Mutation-Positive**



**C EGFR-Mutation-Negative**



L858R mutatie EGFR gen

Abnormaal overactief EGFR eiwit

Overactivatie van MAPK-pathway

Ongecontroleerde celdeling

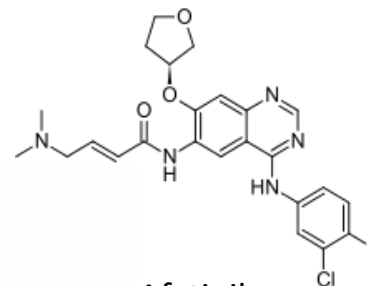
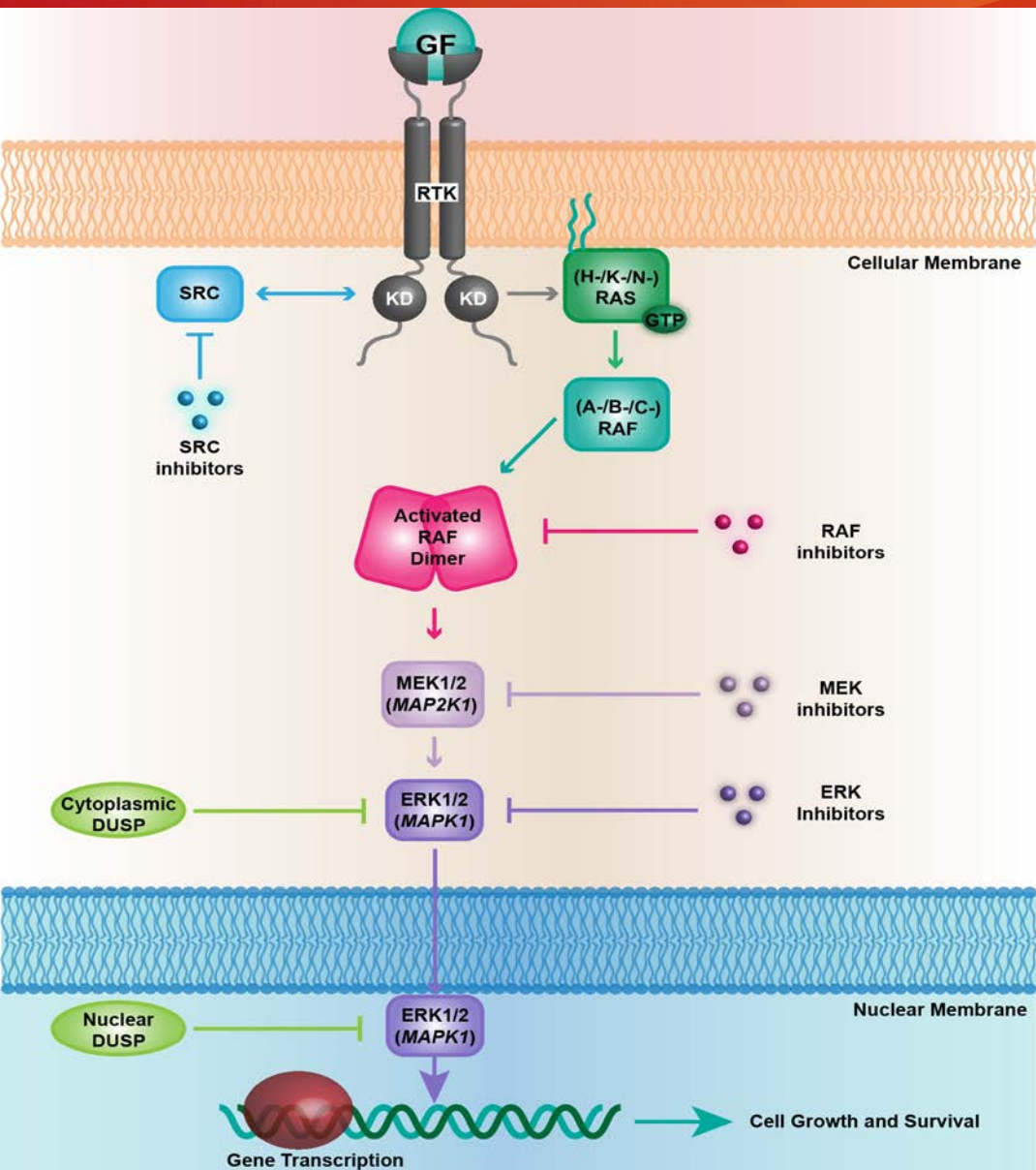
Longkanker

Cel dood tumorcellen

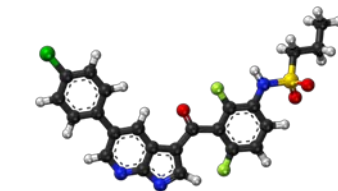
Langere overleving



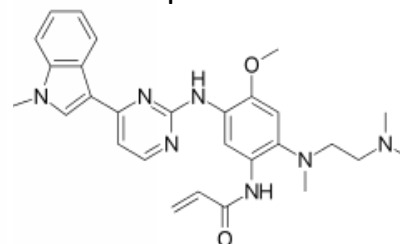
# Precision Medicine



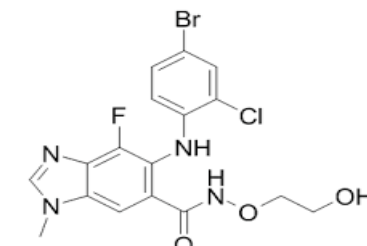
Afatinib  
Spezifiek



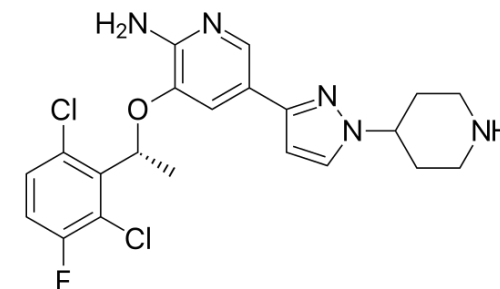
Vemurafenib (Zelboraf®)



Osimertinib (Tagrissa®)  
Spezifiek



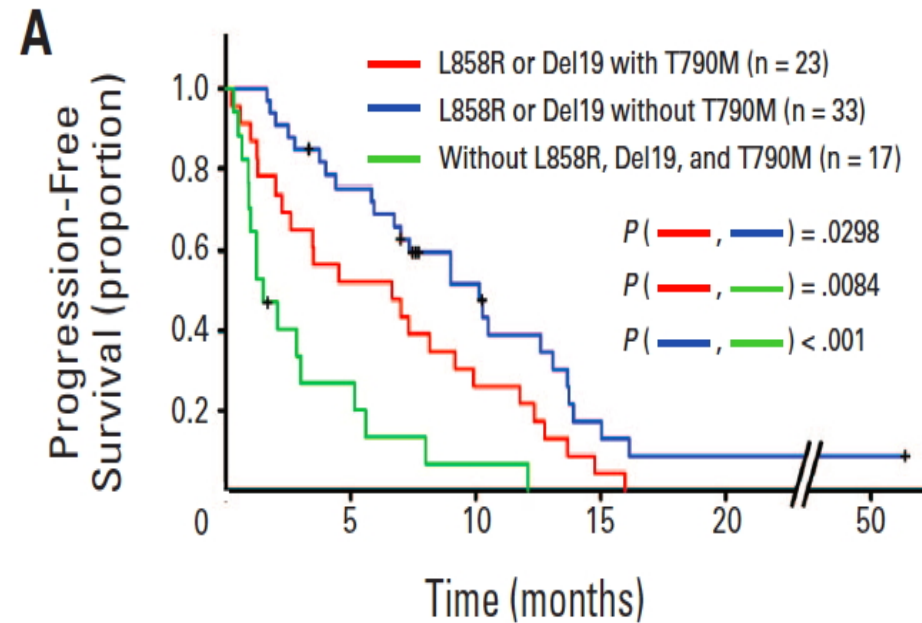
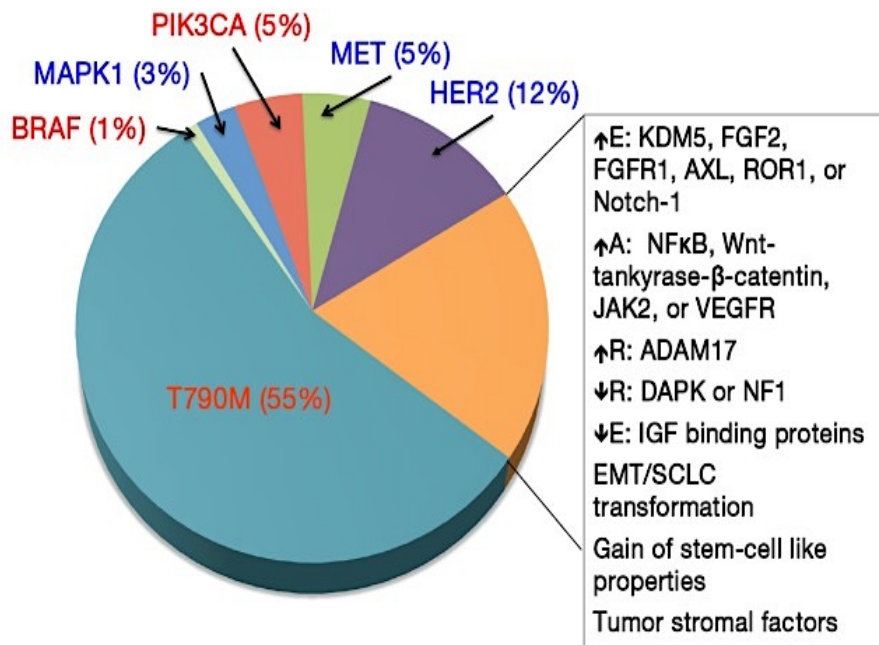
Selumetinib  
Aspezifiek



Crizotinib (Xalkori®)  
Aspezifiek



## Acquired Resistance Mutations: T790M EGFR



## Actionable Mutations



## Relevante Gene Panels

Gen	Locatie (codon/exon)	Alteratie	Frequentie in NSCLC (met mutatie in desbetreffend gen)
EGFR	Exon 18	c.2156G>C (G719A)	0,6%
	Exon 19	Deletie	48%
	Exon 20	Insertie	4-9,2%
	Exon 20	c.2369C>T (T790M)	
	Exon 21	c.2573T>G (L858R)	43%
	Exon 21	c.2582T>A (L861Q)	2%
KRAS	Codon 12	c.34G>T (G12C)	42%
	Codon 12	c.35G>A (G12D)	17%
	Codon 12	c.35G>T (G12V)	20%
BRAF	Exon 11	c.1406G>C (G469A)	11%
	Exon 15	c.1799T>A (V600E)	55%
PIK3CA	Exon 9	c.1633G>A (E545K)	26,7%
	Exon 20	c.3140A>G (H1047R)	12,9%
HER2	Exon 20	Insertie	83-100%
MEK1	Exon 2	c.171G>T of K57N	64%
	Exon 2	c.167A>C of Q56P	19%
MET		Amplificatie	
	Exon 14	Skipping mutaties	3-4%
FGFR1	Chromosoom 8p12	Amplificatie	13-25% van plaveiselcelcarcinoma
ALK	Chromosoom 2p23	Rearrangement	3-7% van NSCLC
ROS1	Chromosoom 6q22	Rearrangement	2% van de NSCLC

## Genomic Landscape of Non-Small Cell Lung Carcinoma

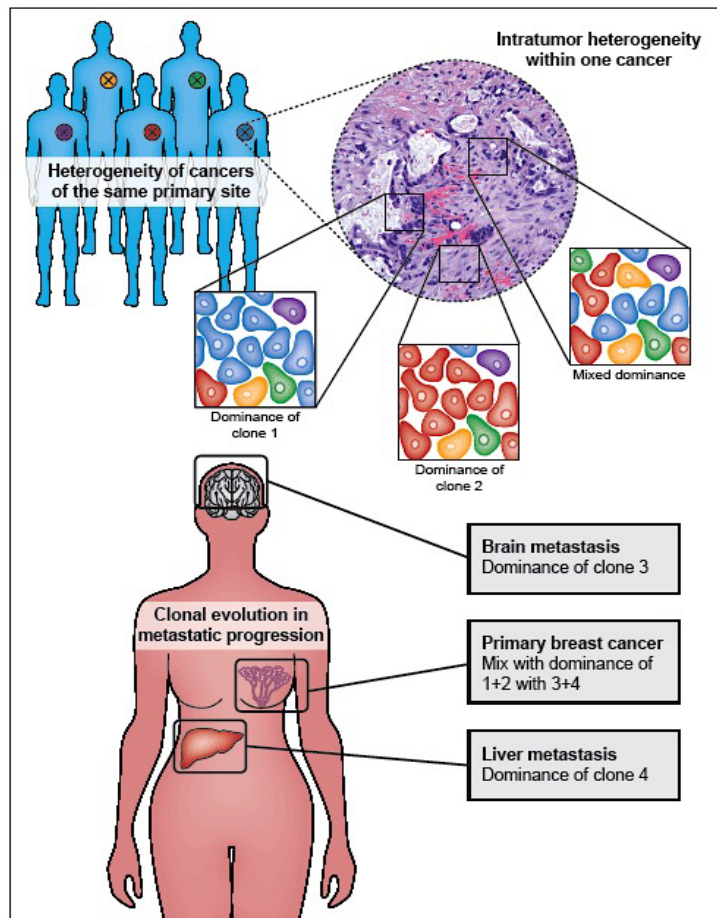
## Beperkingen:

1. Intra-tumorale heterogeniteit
2. Evolutie van therapie-resistente sub-klonen



## Beperkingen:

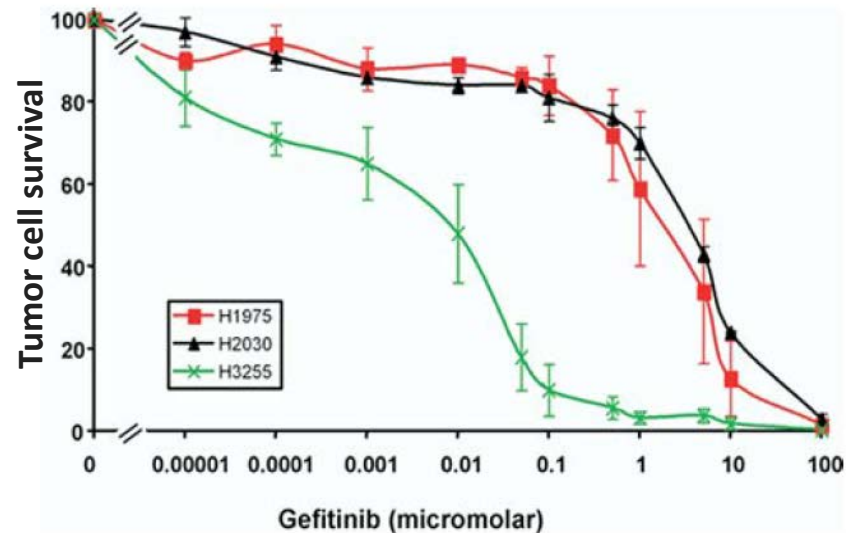
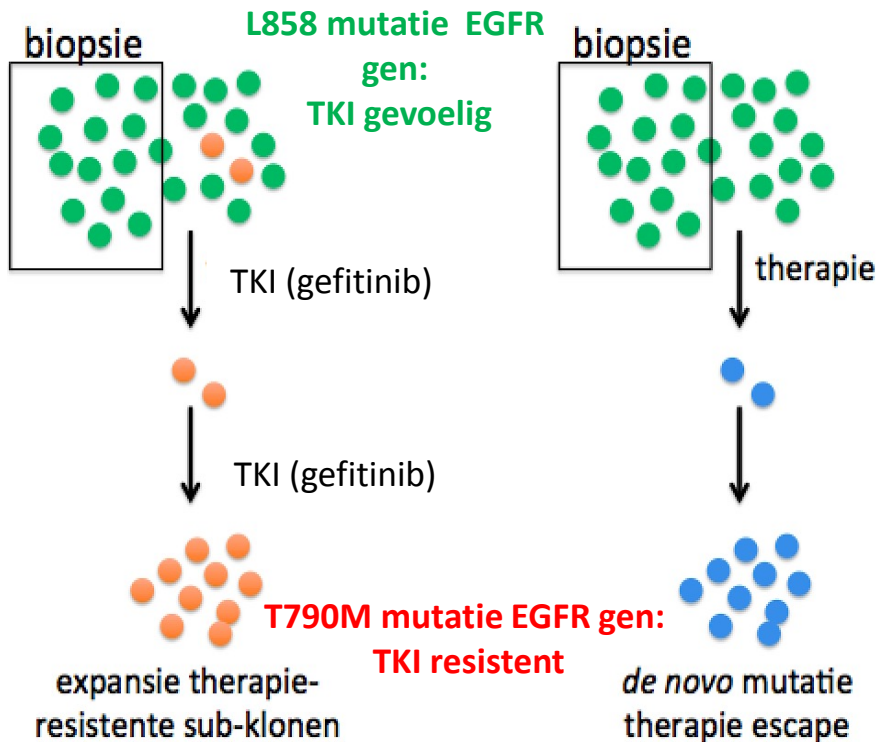
### 1. Intra-tumorale heterogeniteit



- Eénmalige biopsie niet representatief voor gehele tumor
- Puncties (fine-needle aspiration) niet geschikt voor genotypering longtumoren: 42% kans om driver mutatie te missen indien slechts 1 naaldbiopsie wordt geanalyseerd
- Andere beperkingen:
  - Invasiviteit: thoracoscopie/FNA
  - FFPE-geïnduceerde DNA artefacten

## Beperkingen:

1. Intra-tumorale heterogeniteit
2. Evolutie van therapie-resistente sub-klonen



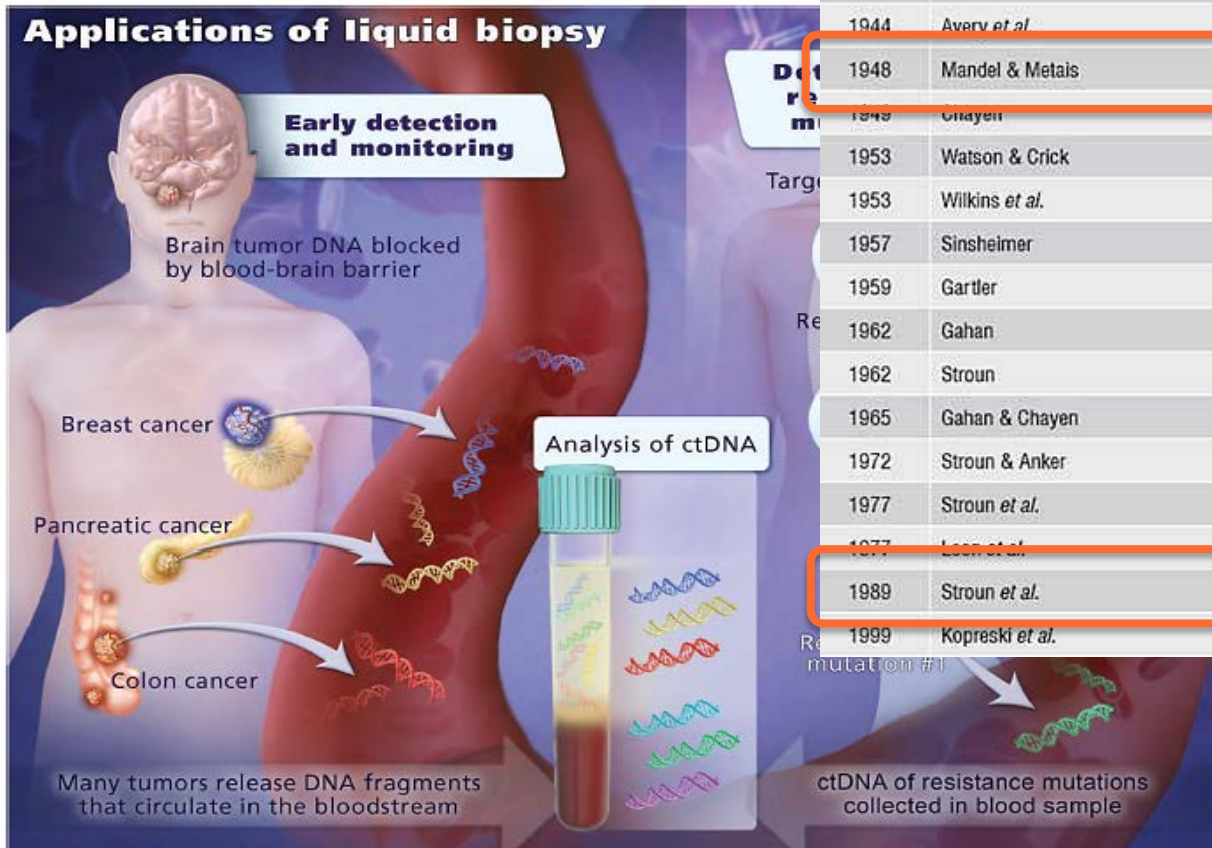
2. Wat is cel-vrij, circulerend tumor DNA (ctDNA) en hoe kan de analyse hiervan een meerwaarde betekenen voor de diagnostiek en opvolging van NSCLC patiënten? Wat is er gekend over de stabiliteit en kritische (pre-) analytische factoren van ctDNA?

1. Wat is ctDNA?
2. Klaring, stabiliteit en (pre-)analytische factoren



# Circulating Tumor DNA (ctDNA)

## Sample Switch!

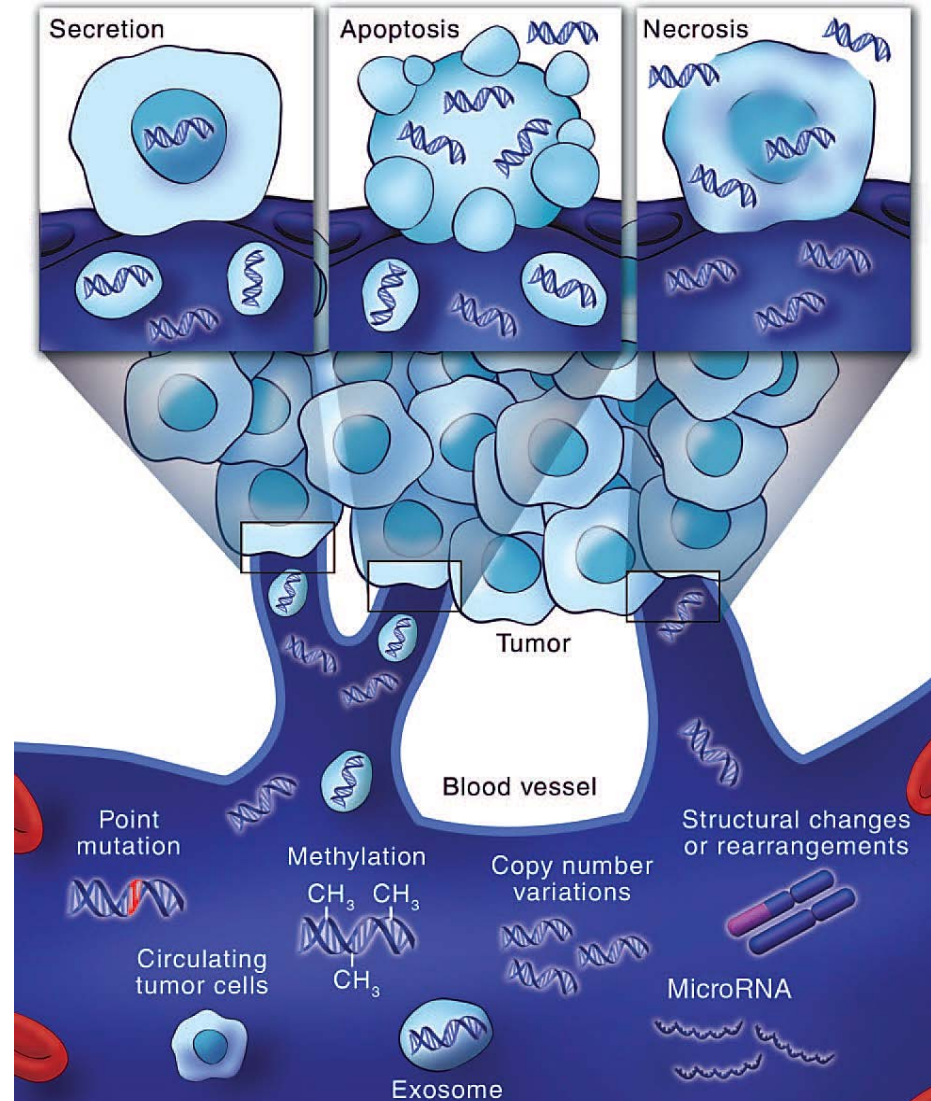


Some developments in understanding DNA and its cellular roles		
Date	Authors	Discovery
1869	Miescher	isolation of DNA (nuclein)
1929	Levene	building blocks of DNA identified
1944	Avery <i>et al.</i>	DNA as genetic material
1948	Mandel & Metals	circulating nucleic acids in blood
1949	Chayen	cytoplasmic DNA localisation
1953	Watson & Crick	DNA structure
1953	Wilkins <i>et al.</i>	DNA structure
1957	Sinsheimer	DNA-gene concept
1959	Gartler	DNA uptake by mammalian cells
1962	Gahan	DNA mobility
1962	Stroun	DNA mobility
1965	Gahan & Chayen	messenger DNA
1972	Stroun & Anker	released nucleic acids
1977	Stroun <i>et al.</i>	circulating nucleic acids
1977	Leen <i>et al.</i>	Blood DNA levels up in cancer
1989	Stroun <i>et al.</i>	cancer derived blood DNA
1999	Kopreski <i>et al.</i>	malignant melanoma serum mRNA



# Circulating Tumor DNA (ctDNA)

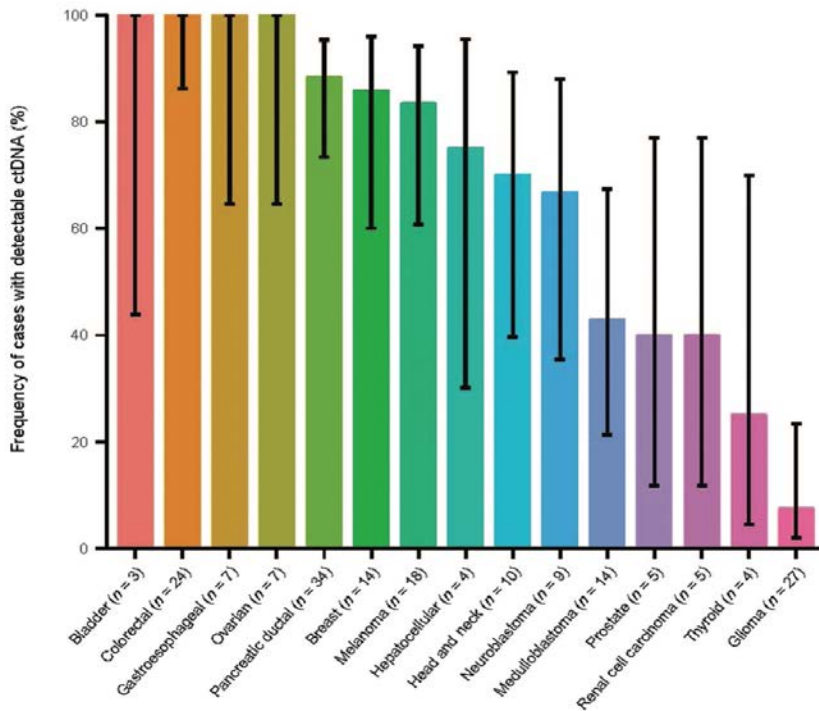
- Meestal <200 bp lang
- ctDNA naast eigen cfDNA
- Vrijgave door:
  - Apoptose
  - Centrale necrose
  - Actieve secretie
- Gemiddelde  $T_{1/2}$ : 15 min - 1.5 u
- Concentratie afhankelijk van:
  - Tumor-type
  - Tumor-load
- Circulerende tumorcellen (CTC)
  - Lagere concentraties
  - Complexere isolatie/detectie



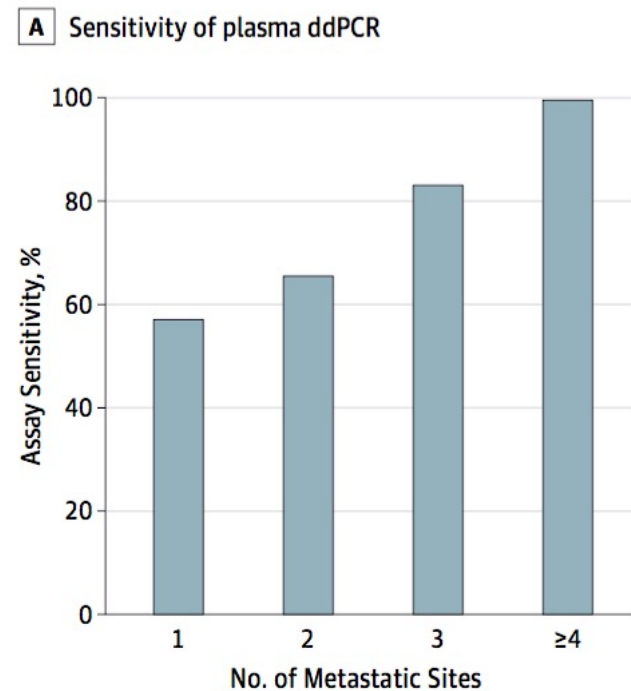
# Circulating Tumor DNA (ctDNA)

- Concentratie, afhankelijk van:

Tumor-type

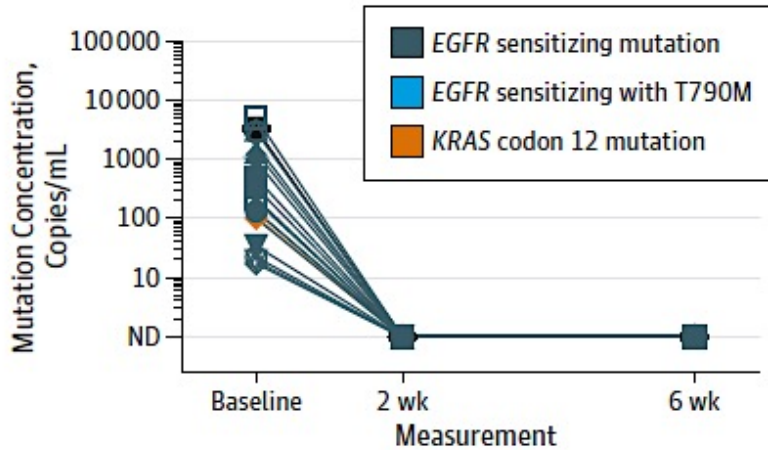


Tumor-load

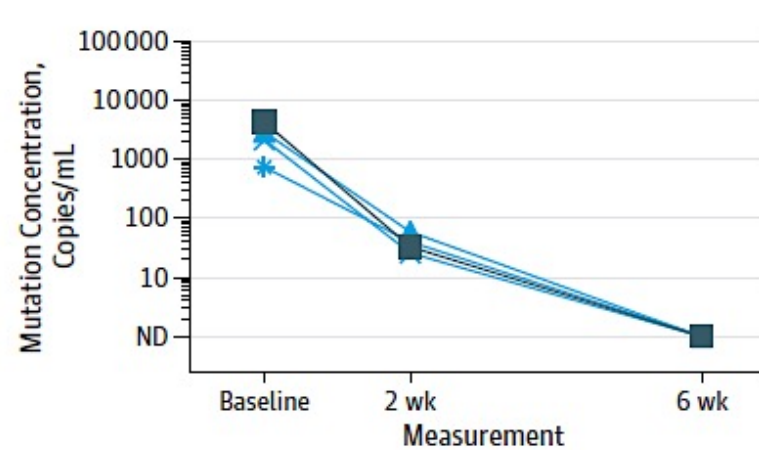


# Circulating Tumor DNA (ctDNA)

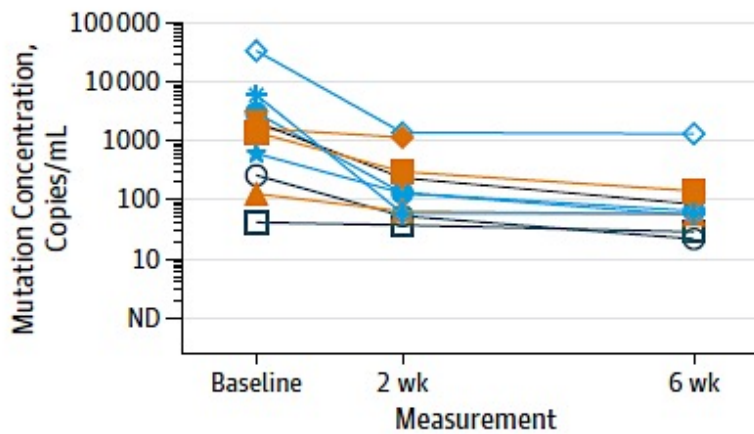
**A** Undetectable at 2 wk



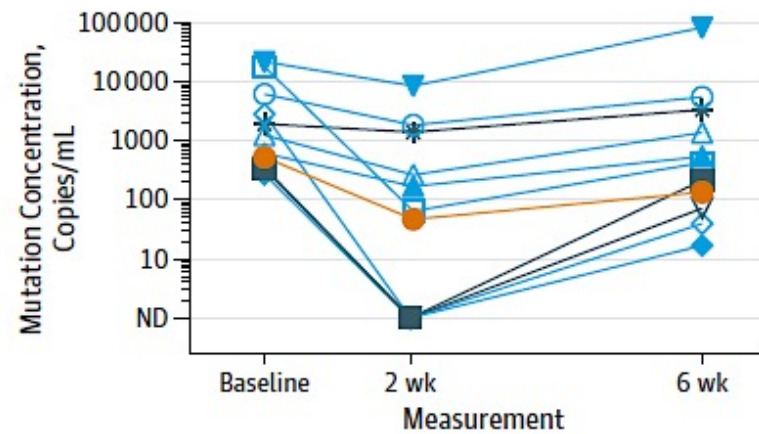
**B** Decrease to undetectable at 6 wk



**C** Decrease but still detectable at 6 wk

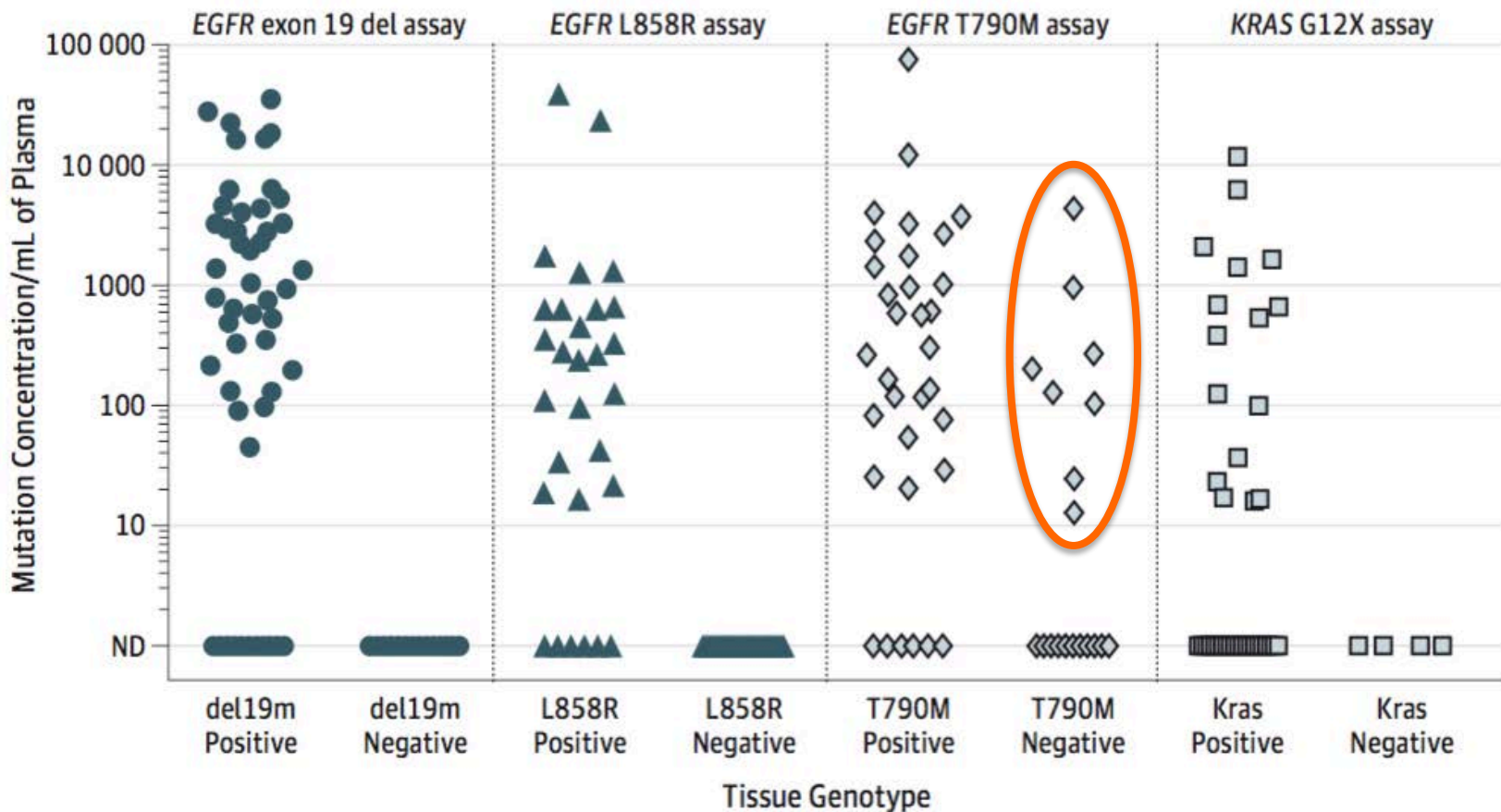


**D** Decrease with rebound



# Circulating Tumor DNA (ctDNA)

**B** Dynamic range of plasma genotyping



3. Welke moleculaire assays kunnen gebruikt worden voor de detectie van ctDNA en met welke technische aspecten moet men rekening houden?

1. Targeted (PCR-gebaseerde) versus untargeted (sequencing-gebaseerde) tests
2. Concordantie/diagnostische performantie van DNA analyse: tumorweefsel versus plasma
3. Klinische meerwaarde van ctDNA analyse?



## Diagnostische Performantie ctDNA?

Technologie	Platform	Tumor type	Patients included	Concordantie	Sensitiviteit	Specificiteit	Referentie
<b>A) Detectie van EGFR mutaties in ctDNA bij NSCLC patiënten</b>							
ddPCR	BioRad	NSCLC, metastatic	180		74-82	100	Sacher A. et al. JAMA Oncol 2016
ddPCR	BioRad	NSCLC, stage III-IV	25		81	100	Zheng D. et al. Sci Rep 2016 (63)
ddPCR	BioRad	NSCLC, stage IV	50	82	76	88	Wei Z. et al. Springerplus 2016 (64)
RT-PCR	Cobas	NSCLC, stage II-IV	199		61	96	Weber B. et al. BMC Cancer 2014
ARMS RT-PCR	Qiagen	NSCLC	652	94	66	100	Douillard J. et al. J Thorac Oncol 2014 (65)
RT-PCR; ARMS RT-PCR; ddPCR; BEAMING	Various	NSCLC, stage IV	38		78-100	93-100	Thress K. et al. Lung Cancer 2015 (66)
NGS	Ion Torrent PGM	NSCLC, stage I-IIA	58	50	54	47	Chen K. et al. Sci Rep 2016 (67)
NGS	Ion Torrent PGM	NSCLC, I-IV	107	68	58	87	Couraud S. et al. Clin Cancer Res 2014 (68)
NGS	Ion Torrent PGM	NSCLC, stage I-IV	288		54	94-98	Uchida J. et al. Clin Chem 2015 (69)
NGS	Resolution Bioscience	NSCLC, advanced	48		79	100	Paweletz et al. Clin Cancer Res 2015 (70)
NGS	Illumina	NSCLC, stage IIIa-IV	39	84	71	100	Yao Y. et al. Oncotarget 2016 (71)
iDES-enhanced CAPP-Seq	?	NSCLC, stage III-IV	142		92	100	Newman A. et al. Nat Biotechnol 2016 (72)
Meta-analysis 27 studies	Mostly PCR	NSCLC			62	96	Qiu et al. Cancer Epidemiol Biomarkers Prev 2015
NGS	Ion Torrent PGM	Various cancers	283		55		Jovelet et al. Clinical Cancer Res 2016**
NGS	Ion Torrent PGM	Various cancers	34		97		Lebofsky R. et al. Mol Oncol 2015** (73)
NGS; BEAMING; ddPCR	Various	Various cancers		40-85			Bettegdowna C. et al. Sci Transl Med 2014**
<b>B) Detectie van KRAS mutaties in ctDNA bij NSCLC patiënten</b>							
ddPCR	BioRad	NSCLC, metastatic	180		64	100	Sacher A. et al. JAMA Oncol 2016
COLD-PCR/HRM; RT-PCR	Various	NSCLC	82	95	96	95	Freidin M. et al. Clin Chem 2015 (74)
NGS	Illumina	NSCLC, stage IIIa-IV	39	97	75	100	Yao Y. et al. Oncotarget 2016

## Keuze van Moleculair Platform?

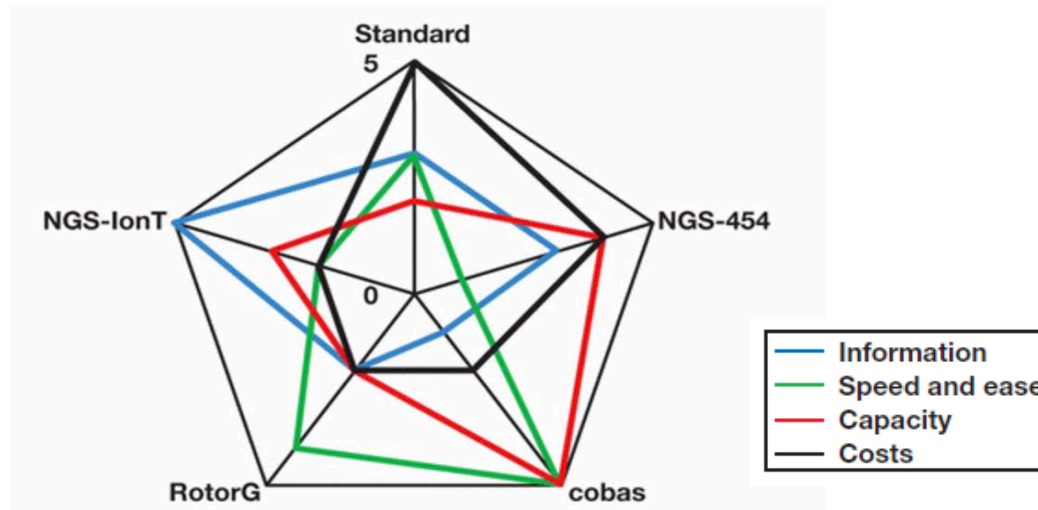


Figure 1

**Table 4**  
**Cost Overview for Combined *KRAS* and *EGFR* Mutation Analysis on Different Platforms<sup>a</sup>**

Cost, €	Standard	NGS-454	NGS-Ion T	cobas	RotorG
Apparatus	140,000	100,000	80,000	45,000	40,000
Costs/sample					
Depreciation apparatus <sup>b</sup>	28	20	16	9	8
Chemicals <sup>c</sup>	20	40	165 (25)	230	220
Labor <sup>d</sup>	26	45	47 (7)	2	20
Total	74	105	228 (48)	241	248

<sup>a</sup> Values in parentheses represent the estimated costs if exclusively *KRAS* and *EGFR* amplicons are analyzed for 96 (maximum commercially available barcodes) samples.

<sup>b</sup> Based on replacement after 5 years and 1,000 samples per year.

<sup>c</sup> Excluding cost for DNA isolation.

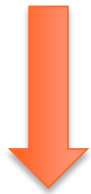
<sup>d</sup> Based on €35 per hour.

- Gevoeligheid ctDNA onvoldoende om weefselbiopsie te vervangen voor **primaire diagnose** (lage sensitiviteit)
- ctDNA heel geschikt om gekende 'actionable mutations' te **monitoren** (zeer hoge specificiteit)
- Klinische meerwaarde ctDNA:
  - Minimaal invasief
  - Representatief voor totale tumorload
  - Snelle bijsturing van precisie therapie mogelijk





- Geen prospectieve studies (progressievrije overleving, totale overleving... )
- Nood aan (pre-) analytische guidelines
- Geen consensus over preferentiële techniek (PCR vs. NGS)



Afwegen: kost, # stalen, TAT, flexibiliteit  
en informatie-capaciteit



Bedankt voor jullie aandacht!

Vragen?

